REMARKS

Reconsideration of this application is requested in view of the amendments to the claims and the remarks presented herein.

Applicants' attorney wishes to thank the Examiner in charge of the application for the courtesies extended to him at the interview on April 29, 2003 when the final rejection was discussed.

The claims remaining in the application are claims 25, 28 to 30, 33, 34 and 36, all other claims having been cancelled.

All of the claims stand rejected under 35 USC 112, second paragraph, as being indefinite. The Examiner objected to the term "avoiding cardiovascular disease" as being indefinite. The deletion of this term from the claims obviates this ground of rejection.

All of the claims were rejected under 35 USC 112, first paragraph, as being based upon a specification that was not enabling for the present claims. The Examiner indicated that the specification was enabling for administering the combination of nomegestrol and estradiol in a continuous or intermittent fashion from 21 to 25 days but did not reasonably provide enablement for "continuously without interruption". The Examiner further objected to the specification as not being enabling for estradiol esters or equine conjugate estrogens.

Applicants respectfully traverse these grounds of rejection since it is deemed that the claims are clearly enabled by the present specification. With respect to the continuous administration without interruption, lines 4 to 6 of page 4 indicate that "the compositions according to the invention based on nomegestrol and free or esterified estradiol or equine conjugated estrogens are administered in a continuous or intermittent fashion from 21 to 25 days per month." Moreover, it is clear that the specification provides two alternatives of administration, one without interruption and another one 21 to 25 days.

As to the length of the administration, the examples disclosed periods of at least 24 weeks. Example 2 of the specification describes a clinical trial in which an estradiol/nomegestrol acetate composition is given continuously and without interruption every day to post-menopausal women for at least 24 weeks with the treatment being extended to 48 weeks for some women. It is worth noting that the women treated for 48 weeks experienced no bleeding. Therefore, clearly, the specification is enabling for continuous administration without interruption and without bleeding. Therefore, the specification is enabling for the present claims.

With respect to the Examiner's speculation as to the unlimited time in claim 34, the specification clearly supports administration of nomegestrol acetate and an estrogen for at least 48 weeks and this can be continued as long as the patient and the doctor consider the same to be efficient and useful.

With respect to Examiner's enabling rejection, it should be noted that the estrogen is selected from a group of free estradiol, esterified estradiol and conjugated equine estrogens. The examples describe a combination of nomegestrol acetate and either free estradiol or estradiol valerate which is an ester of estradiol. In the office action of February 11, 2000, the Examiner acknowledged that estradiol valerate would be expected to have the same properties as estradiol and it is further well established that estradiol, estraiol esters and conjugated equine estrogens fall in the same therapeutic class, i.e. natural steroidal estrogens, they have equivalent effects on the reproductive system and menopause symptoms. Applicants are submitting herewith a copy of an article entitled "Estrogen Replacement and Coronary Heart Disease" by Barret-Connor et al. Moreover, "equine conjugated estrogens" is a well known term of the art represented by the product Premarin of a known composition and can properly be described as a generic term for the product and a representative dosage is given in the application as filed (see page 3, line 25; page 4, line 13 and page 6, line 17). Therefore, the claims are fully enabled with respect to the estrogen compounds and withdrawal of these grounds of rejection is requested.

As a result of the interview, it was the understanding that the only rejections remaining are those based on Plunkett et al, Fraser et al and Lanquetin et al because the Examiner stated in the first full paragraph of page 2 of the office action that the art rejections were maintained. This was confirmed at the interview.

Applicants respectfully traverse these grounds of rejection since the references in no way anticipate or render obvious Applicants' invention. Applicants are submitting herewith a declaration by Dr. Thomas which explains why the claimed method is not obvious in view of the references. It should be noted that the Lanquetin et al reference has a February 7, 1997 French publication date while the present application has a Frnech priority date of October 8, 1996 and Applicants state for the record that the present application is a complete English translation thereof and therefore, Lanquetin et al is not part of the prior art for the present invention.

To summarize the declaration, Dr. Thomas states that Fraser et al does not teach or suggest using nomegestrol acetate "continuous without interruption" therapy and only gives a short term treatment with interruption which induces bleeding. The same is true for the Lanquetin et al reference and the Examiner's attention is directed to the conclusion of the Fraser et al reference as not teaching a continuous and non-interrupted combination of nomegestrol acetate and an estrogen.

With respect to the Plunkett et al reference, this fails to disclose nomegestrol acetate as the progestin and there is a distinct difference between the progestin of Plunkett et al and that of the nomegesterol acetate. The Examiner's attention is directed to page 4 wherein the differences between nomegestrol acetate and the progestins of Plunkett et al are clearly discussed.

As noted continuously throughout the prosecution of the application, Applicants' invention relates to the administration of both the estradiols, the esters thereof or the equine conjugated estrogens and nomegesterol acetate continuously and without interruption. Table 3 also shows the pharmacological profile of the nomegestrol acetate v. other progestins and clearly demonstrates the differences between the two. Figure 2 also shows the endometrial effects of the estradiol and nomegestrol acetate continuous combination. Therefore, the prior art does not teach Applicants' invention and the advantages thereof and withdrawal of these grounds of rejection is requested.

In view of the amendments to the claims, the declaration of Dr. Thomas and the above remarks, it is believed that the claims clearly point out Applicants' patentable contribution and favorable reconsideration of the application is requested.

Respectfully submitted,
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CAM:ds
Enclosures

MARKED UP VERSION OF CLAIMS SHOWING CHANGES MADE

Claim 34 (thrice amended) A method of treating estrogenic deficiencies in women while further avoiding the appearance of osteoporosis, withdrawal bleeding and cardiovascular diseases in post-menopausal women without any androgenic effect, and no deleterious effects on blood vessels comprising continuously without interruption administering to said women, a combination of 0.5 to 3 mg of an estrogenic compound selected from the group consisting of free or esterified estradiol or equine conjugated estrogens and 1.5 to 3.75 mg of nomegestrol acetate.

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CHAPTER 10

Estrogen Replacement and Coronary Heart Disease

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Myocardial infarction is uncommon in premenopausal women in the absence of some predisposing condition such as diabetes or familial hypercholesterolemia. However, women who are oophorectomized before the age of natural menopause appear to have an increased risk of atherosclerosis. These observations suggest that endogenous estrogen is protective: if so, exogenous estrogen as partial replacement therapy for loss of ovarian function might be protective also. The implications of this possible benefit are large: coronary heart disease is far and away the leading cause of death in postmenopausal women.

We review here selected clinical data with regard to the effect of estrogen replacement therapy on heart disease risk factors and observational studies with regard to estrogen replacement therapy and heart disease. Because sequential progestin is now frequently added to estrogen therapy (to reduce the risk of endometrial cancer), the effects of estrogen alone are contrasted with estrogen plus progestin regimens wherever data permit.

THE HORMONES

Estrogen

Some of the discrepancies in the literature about the effects of estrogen replacement therapy on heart disease risk factors result from a failure to consider the different estrogens and progestins used. Equivalent effects on the reproductive system and/or menopause symptoms do not necessarily equate with similar effects on heart disease risk factors. For cyclic or sequential regimens, the timing of the measurements or venipunctures may be important also.

Estrogens used for therapeutic purposes can be divided into three major classes: (1) the "natural" steroidal estrogens such as conjugated equine estrogens (Premarin), 17-β-estradiol (Estrace), and estrone sulfate (Ogen); (2) the synthetic steroidal estrogens, including ethinyl estradiol (Estinyl) and mestranol; and (3)

the synthetic nonsteroidal formulations, including stilbestrol and diethylstilbestrol (DES). "Natural" estrogens are distinguished from synthetic ones by the fact that their chemical structures are found in nature (although not necessarily in humans), whereas the chemical structures of the synthetic agents are man-made.

Unlike the formulations used for contraceptive purposes, in which synthetic estrogens are used exclusively, nearly all of the estrogens prescribed for menopausal symptoms are natural. In the United States, Premarin alone accounts for 75 percent of all prescriptions, and other natural agents account for approximately 15 percent of use. Synthetic estrogens, particularly ethinyl estradiol, make up the remainder of use for menopausal replacement therapy.

The relative potency of synthetic compared with natural compounds is variable and highly dependent on the target tissue. Thus, it is difficult to assess in any systematic manner. If the ability to suppress ovulation is considered, the potency of the usual dose of Premarin (0.625 mg) is between 10 and 40 percent that of the usual doses of ethinyl estradiol (30 to 50 μg). The synthetic estrogens also appear to have greater impact than natural agents on coronary risk factors, including blood pressure, lipids and lipoproteins, glucose tolerance, and clotting parameters. However, insufficient data exist to address adequately these latter relationships.

Progestins

Progestins can be grouped into three categories: two types of synthetic formulations, and natural progesterone. The two major classes of synthetic progestins available are the 19-nor-testosterone (19-nor) derived hormones, which include norethindrone (norethisterone), norethindrone acetate (Norlutate), and levonorgestrel (Ovrette), and the C-21 progestins derived from 17-alpha hydroxyprogesterone, including hydroxyprogesterone caproate (Prodrox) and medroxyprogesterone acetate (Provera). The 19-nor agents are used exclusively in combination-contraceptive therapy, and have been shown to have strong androgenic properties. The 17-alpha agents, in particular Provera, are usually used in menopausal replacement therapy and are considered less androgenic than the 19-nor agents. In the United States, approximately 90 percent of women receiving hormonal replacement therapy use Provera, and the remaining 10 percent use norethindrone acetate. Recently, natural progesterone has been micronized for oral use and appears to have little or no androgenic effect. It has not been studied extensively and is not commercially available in the United States.

Although some progestins do have estrogenic effects, the major metabolic effects of progestational agents appear to be dependent on estrogen priming; that is, progestins behave primarily as antiestrogens by blocking the synthesis of new cytoplasmic estrogen receptors. Because of this metabolic symbiosis, biologic effects of unopposed progestins have not been systematically evaluated.

A variety of studies have evaluated the effects of various estrogen formulations and regimens in relation to risk factors. However, almost all of the estrogen–heart disease studies have involved estrogen replacement therapy with unopposed (i.e., without progestin) conjugated equine estrogen (Premarin). Extrapolation of these study results to other estrogens or to estrogen plus progestin regimens is not necessarily valid.

ESTROGEN REPLACEMENT THERAPY AND HEART DISEASE RISK FACTORS

Obesity

Cross-sectional population-based data suggest that women given postmenopausal estrogens are leaner than those not so treated,[1,2] but do not exclude the possibility that thin women are more likely to be prescribed estrogen replacement therapy. The few trials of sufficient duration to address the effect of estrogen replacement therapy on weight suggest that estrogen may modify weight gain. Hart and coworkers[3] reported that overweight oophorectomized women treated with 40 μg/day of mestranol for 1 to 7 years tended to lose weight, whereas overweight placebo recipients gained weight; no change in weight was noted in normal-weight women with or without estrogen replacement therapy. Similarly, Jensen and coworkers[4] reported that body weight did not change in postmenopausal women treated for 1 or 2 years with percutaneous estrogen, but there was significant weight gain in women treated with placebo.

Blood Pressure

Contrary to the literature on oral contraceptives, most studies suggest that the majority of estrogen-treated postmenopausal women experience a reduction in blood pressure with estrogen replacement therapy. Differences in reported estrogen–blood pressure associations may reflect different hormone products, doses or duration of use, small sample size, subject selection, and/or the limited number of blood pressure measurements made before and during treatment. In addition, observations often were made by persons without training in standardized blood pressure measurement, and relatively few investigators considered the effect of pretreatment blood pressure on the results.

In one of the better clinical trials, Lind and associates[5] recruited 56 women aged 49 to 55 years from general practices into a randomized placebo-controlled study of three available forms of oral estrogen (conjugated equine estrogen, 1.25 mg/day; piperazine estrone sulfate, 1.5 μg/day; and estradiol valerate, 2 mg/day), each given with or without the progestin norgestrel, 0.5 mg. Although there were relatively few women in each treatment group, each had several measurements of blood pressure before, during, and after hormone replacement therapy. Overall, these women had statistically significant decreases in both systolic and diastolic blood pressures, which returned to pretreatment levels after replacement therapy was discontinued. No difference in response was mentioned when estrogen was prescribed with a progestin. Approximately one-fourth of all treated women had no change in their blood pressure.

In another randomized clinical trial, Luotola[6] treated 20 normotensive and 20 hypertensive women, aged 41 to 55, who were seen for menopause symptoms with 2 or 4 mg/day of 17-β-estradiol. In this cross-over design, both normotensive and hypertensive women had significant reductions in systolic and diastolic blood pressures, which reversed during the placebo period. These blood pressure changes correlated significantly with changes in serum estrone. No other clinical or physiological characteristic distinguished these women from the few patients who had a modest *rise* in blood pressure with estrogen replacement therapy or explained the fall in blood pressure in the majority.

A third randomized clinical trial, by Wren and Roultedge,[7] included a much larger number of patients referred from a menopause clinic, but obtained only a single pre- and post-treatment blood pressure. Each patient received 24 days of one of two oral estrogens and 0.03 mg of levonorgestrel on days 15 to 24. There was a consistent decrease in both systolic and diastolic blood pressures in the 184 women assigned to piperazine estrone sulfate, 0.625 to 1.25 mg/day. No blood pressure change was observed in 144 women who received conjugated equine estrone in doses of 0.3 to 1.25 mg/day. In this study, no dose response effect was seen with either product.

The observation that oral, but not percutaneous, estrogen is associated with an increase in renin substrate is probably unrelated to the observed blood pressure effects. The long-term effect of oral and percutaneous estrogen replacement therapy on blood pressure and plasma renin was studied by Hassager and associates[8] in a 2-year placebo-controlled study of 110 early postmenopausal women. In this study, women were allocated to one of four treatment groups: oral cyclical combination of 2 mg estradiol valerate and cyproterone acetate; oral placebo; percutaneous 17-β-estradiol, supplemented by 200 mg of oral progesterone during the second year; and percutaneous placebo cream. Systolic and diastolic pressures remained unchanged in both estrogen treatment groups, whereas a significant increase in diastolic blood pressure was observed in both placebo groups. Plasma renin substrate increased during oral treatment with estradiol but was unchanged with percutaneous estradiol; no correlation was found between blood pressure and plasma renin substrate.

Synthetic progestins have been implicated in hypertension from the studies of oral contraceptives. However, natural progesterones have vasodilating properties. In a double blind study of four hypertensive postmenopausal women by Rylance and colleagues,[9] micronized progesterone was alternated every 2 weeks with placebo, and the dose was increased from 200 mg/day to a total of 600 mg/day. There was a significant fall in blood pressure while the women were receiving progesterone, but not while taking placebo. The maximum fall coincided with the highest dose, an average of 19.7 mmHg systolic and 9.6 mmHg diastolic. In the aforementioned study by Hassager and colleagues,[8] however, diastolic and systolic blood pressures and renin substrate were not influenced by the addition of micronized progesterone to oral or percutaneous estrogen.

These studies suggest that estrogen replacement therapy has no adverse effects on blood pressure levels in the majority of women, in whom it may, in fact, be hypotensive.

Clotting Factors

Large doses of estrogen may alter clotting factors and increase the risk of thrombotic events in premenopausal women. However, studies of clotting and estrogen replacement therapy in postmenopausal women are rare. In an older report, Bonnar and coworkers[10] performed serial studies of coagulation factors in three small groups of women with menopausal symptoms. Eleven women who received large doses of mestranol (up to 50 μg) and norethisterone, 1.5 mg/day, had increases in Factors VIII, IX, and X and a decrease in antithrombin III. Both estradiol valerate (2 mg/day) and conjugated equine estrogen (1.25 mg/day) increased Factor VII and X complex, but only the former increased Factors II and X. Neither had a measurable effect on antithrombin III. Hart and coworkers[3]

compared clotting function in 146 women taking mestranol and 121 taking placebo for 1 to 7 years. There was no significant difference in prothrombin time, partial thromboplastin time, or Factor X, but 24 patients taking mestranol (in an average daily dose of 25 μg) had elevated Factor VIII compared with 7 women in the placebo group.

In more recent reports, there is less evidence of abnormal clotting with estrogen replacement therapy. In the randomized clinical trial reported by Lind and associates,[5] none of the six estrogen replacement therapy regimens (detailed above under Blood Pressure) resulted in a change in antithrombin III, prothrombin time, partial thromboplastin time, fibrogen degradation products, Factor V, VIII, or X, platelet count, or platelet aggregation. More recently, Chetkowski and colleagues[11] reported no change in fibrinogen A, high molecular weight fibrogen, antithrombin III level, or activity in 23 women randomly assigned to up to 200 μg/day of transdermal estradiol or up to 1.25 mg/day of conjugated equine estrogen.

The earlier reports of adverse coagulation effects associated with hormone replacement may have been due to a very high dose of estrogen or the concurrent use of a progestin. No hormonal effect on blood coagulation is the rule with current regimens.

Lipids and Lipoproteins

In contrast to the paucity of studies of estrogen replacement therapy on obesity, blood pressure, and coagulation, multiple studies of estrogen replacement therapy with regard to lipids and lipoproteins have been reported. Despite the large number of studies, repeated investigations of the same estrogen or estrogens in the same dose and regimen to women with similar treatment eligibility criteria are rare, and this plus small sample size in many studies precludes a meaningful comparison or synthesis of these data. The studies briefly reviewed here were selected because of superior design, because of illustration of a particular point, or because they are the only available studies of a particular regimen.

A great many studies suggest that estrogen replacement therapy has little or no effect on total plasma cholesterol and a variable effect on triglycerides.[12] The triglyceride elevating effect of synthetic and equine estrogens is presumably due to increased production. As reviewed elsewhere,[13] androgenic progestins such as norethindrone probably lower triglyceride in women whose hypertriglyceridemia is due to increased production. The thesis that nonalkylated estrogens like estradiol valerate have no effect on triglyceride or very low density lipoprotein (VLDL)[14] has not been confirmed by all investigators.[15] Since the relationship of triglyceride to coronary heart disease risk is controversial,[16] and any effect may be mediated via the inverse association of high density lipoprotein (HDL) with triglyceride, the remainder of this review will focus on the lipoproteins. Obviously, the proportionate effects of estrogen replacement therapy on HDL and low density lipoprotein (LDL) will also determine to a large extent the overall effect on total cholesterol.

Since the 1952 report by Barr and coworkers[17] that oral estrogen therapy increases alpha lipoprotein (HDL) and decreases beta lipoprotein (LDL), nearly all studies have confirmed that unopposed oral estrogen causes lower LDL and higher HDL levels, i.e., a favorable lipoprotein ratio with regard to heart disease risk. The range of reported responses probably reflects the effect of different dose,

drug, and duration of therapy, cyclic versus continuous use, and/or subject selection, sampling frame, and sampling schedule. For example, the use of conjugated equine estrogens, the most popular non-contraceptive estrogen in the United States, has been associated with lower LDL and higher HDL levels in most studies. As reviewed by Bush and Miller,[12] the broad range of reported values includes a 0 to 26 percent increase in HDL and a 4 to 19 percent decrease in LDL; after correcting for the size and duration of the study, at a 0.625-mg daily dose, HDL levels are increased by 10 percent and LDL levels are decreased by 4 percent. With a higher 1.25-mg dose, HDL increased by 14 percent and LDL decreased by 8 percent.

In contrast to the general agreement that unopposed oral estrogens raise HDL and lower LDL cholesterol, there is more controversy about the effect of parenteral and percutaneous estrogen. Reported differences in lipoprotein levels are not entirely explained by dose or route of administration. Fletcher and coworkers[18] studied 34 bilaterally oophorectomized women who received 50 mg 17-β-estradiol by subcutaneous implant every 6 months. Compared with 67 untreated oophorectomized women, there was no significant difference in HDL (including subfractions) or LDL levels in the treated women after 6 months or again after 3 years, despite their high serum estradiol levels. In another study, however, there was a significant fall in LDL and rise in HDL levels 14 weeks after implantation of a larger dose of 100 mg of 17-β-estradiol in eight oophorectomized women.[19] With transdermal estradiol in doses up to 200 μg/day, Chetrowski and associates[11] reported no significant change in LDL and HDL. However, Jensen and associates[4] treated 45 postmenopausal women for 2 years with either 3 mg of percutaneous estradiol or placebo. In this study, percutaneous estradiol significantly reduced LDL but had no effect on HDL. (Addition of micronized progesterone did not ablate these changes and may have raised HDL slightly.) Conjugated equine estrogens given vaginally in doses up to 2.5 mg are reported to have no effect on lipoproteins.[20]

Several investigators have attempted to determine the effect of an added progestin on estrogen-associated lipoprotein changes. In one of the first clinical trials designed to specifically study the effect of different progestins on lipoproteins during postmenopausal therapy, Hirvonen and colleagues[21] treated 18 postmenopausal women with estradiol valerate 3 mg/day for 3 weeks; they were then assigned (in groups of six) to two cycles of norethindrone acetate 10 mg/day, medroxyprogesterone 10 mg/day, or norgestrel 0.5 mg/day. HDL cholesterol decreased by 20 percent in those receiving estradiol plus norethindrone or norgestrel but did not change significantly in the group receiving estradiol plus medroxyprogesterone.

Farish and coworkers[22] treated 21 oophorectomized women with conjugated equine estrogen alone and 21 women who had a natural menopause with conjugated equine estrogen 0.625 mg, plus norgestrel 0.15 mg/day, for the last 12 days of each treatment cycle. Women treated with the unopposed estrogen had a significant increase in HDL, especially HDL_2, and a significant decrease in LDL, whereas those who received both hormones showed only a significant decrease in LDL.

Ottosson[23] studied 140 women, aged 32 to 70, who were treated with three cycles of unopposed oral estrogen followed by the sequential addition of a progestin for the next three cycles. HDL_2 levels were increased by 29 percent on 10 μg of ethinyl estradiol compared with 16 percent on 2 mg/day of estradiol valer-

ate. The addition of synthetic progestin decreased HDL and HDL_2 levels; for example, estradiol valerate given with sequential levonorgestrel reduced HDL_2 by 28 percent, and given with sequential medroxyprogesterone, by 17 percent. In contrast, 200 mg of micronized progesterone had no apparent effect on plasma HDL.

In one of the largest studies, Christiansen and associates[24] randomly allocated 177 postmenopausal women aged 44 to 59 to one of the three daily doses of micronized estrogen in combination with norethisterone 1 mg/day, given from the 13th to 23rd of the month. Over a 3-year period, blood samples obtained every 3 months during the progestin phase showed a 10 to 13 percent reduction in total cholesterol on the high (4-mg) estrogen dose, a 5 percent reduction on the medium (2-mg) dose, and a 3 percent reduction on the low (1-mg) dose. Reduction in total cholesterol was due entirely to reduced LDL cholesterol; there were no significant changes in HDL.

Lipoprotein levels may vary with the timing of the blood sampling in estrogen-progestin treated women. Teichmann and associates[25] studied 20 oophorectomized women before and after 1 year of treatment with 1.25 mg of conjugated estrogen and 5 mg of medroxyprogesterone in a cyclic protocol. Blood obtained on the last 3 days of the cycle showed a significant increase in HDL and a significant decrease in LDL.

Jensen and coworkers[26] studied 30 women aged 45 to 54 who were randomly allocated to high, medium, or low dose micronized estrogen, sequentially combined with norethisterone 1 mg/day, given on days 13 to 22 of two consecutive cycles. Blood for lipid and lipoprotein analysis was obtained twice a week in these women. The lowest total cholesterol was achieved during the estrogen-progestin days, but the lowest HDL was observed during the first 14 days, when estrogen was given alone.

Vejtorp and coworkers[27] randomly allocated 30 perimenopausal women from general practice to receive sequential therapy with either estradiol valerate 2 mg/day and norgestrel 0.5 mg/day, or micronized estradiol 2 mg/day and medroxyprogesterone 10 mg/day. Blood obtained during the estrogen phase showed no difference in lipoprotein level expressed as a percentage of pretreatment level, but blood obtained in the progestin phase showed the percentage of HDL (and VLDL) to be significantly higher in women treated with estradiol plus medroxyprogesterone.

In summary, it appears that unopposed oral estrogen provides an improved lipoprotein ratio, with a more striking effect on HDL than LDL. Results are less consistent with estrogen given by other routes or in conjunction with a progestin. If a progestin is added, the least androgenic preparation and the lowest dose known to inhibit endometrial hyperplasia should be used.

EXOGENOUS ESTROGEN USE AND CORONARY HEART DISEASE

Background

In the early 1960s the concept of long-term estrogen replacement therapy ("feminine forever") was popularized by Wilson and Wilson. From that time until about 1975, millions of American women took unopposed estrogen therapy for prolonged periods, allowing for the observations of long-term sequelae. In

1975, the *New England Journal of Medicine* published two articles showing that unopposed estrogen therapy increased the risk of endometrial carcinoma. Since the publication of those and subsequent articles, unopposed estrogen therapy became less popular. Toward the end of the 1970s, there was sufficient evidence to show that the addition of a progestational agent to an estrogen regimen could negate the increased risk of endometrial carcinoma. Subsequently, most women with intact uteri are prescribed estrogens cycled with progestins.

With the exception of one study reported below (Nachtigall et al), all of the studies reviewed preceded the widespread use of estrogen plus progestin in postmenopausal women. Thus, the vast majority of hormone users were women who took unopposed estrogens. Therefore, the question of the effects of estrogen/progestin therapy on risk of coronary heart disease has yet to be addressed, and the results reviewed here cannot be equated with those that would follow estrogen cycled with a progestin.

Study Results: Overview

Currently there are 19 studies reported (Table 10–1) that have evaluated the effects of estrogen replacement therapy on risk of coronary heart disease.[24–46] Of these 19 reports, 10 are cohort studies,[37–46] 8 are case-control studies,[29–36] and 1 is a randomized clinical trial.[28] Eleven of the 19 reports, including the clinical trial, 8 of the 10 cohort studies, and 2 of the 8 case-control studies, found that women using estrogens had a reduction in the risk of coronary heart disease of 50 percent or greater. Four of the studies (one cohort, three case-control) reported a reduction of risk of coronary disease of 30 to 50 percent in estrogen users. Two reports (both case-control) found no difference in risk for estrogen users, and two studies (one cohort and one case-control) actually found an increased risk of heart disease in women reporting estrogen use.

The variability of these results may be explained by a variety of factors that differed among the studies, including actual study design, study population, age of study subjects, definition of estrogen use, and endpoints considered. Nonetheless, it seems clear that the vast majority of studies to date (~80 percent) have found that estrogen use protects against coronary disease. This protective effect is biologically plausible, inasmuch as estrogens have marked beneficial effects on lipids and lipoproteins, and apparently do not adversely affect other risk factors for CHD.

Study Results: Review of Studies

Clinical Trials

Only one clinical trial of estrogen use and risk of coronary disease has been published. Nachtigall and colleagues[28] reported in 1979 the results of a double-blind randomized trial of 10 years' duration. Participants were residents of a long-term care chronic disease hospital, and most suffered from chronic conditions such as diabetes mellitus, neurologic disorders, and arteriosclerosis. Eighty-four age- and condition-matched pairs of women were selected for the trial, and one woman of each pair was randomly assigned to take 2.5 mg of Premarin daily and 10 mg of Provera for 7 days a month. The other half of the pair took placebos. At the end of 10 years of follow-up, women assigned hormonal therapy, compared

Table 10–1. Summary of Studies of Replacement Estrogen and Cardiovascular Disease

Study	Study Design	Population Size	Endpoints	Relative Risk	p Value
Nachtigall et al[28]	Randomized trial	84 pairs	Fatal/non-fatal MI	0.33	p >.05
Talbott et al[29]	Case-control	64 cases 64 controls	Sudden death	0.34	p >.05
Ross et al[30]	Case-control	133 cases 133 controls	Fatal CHD	0.43	p <.01
Szlko et al[31]	Case-control	36 cases 39 controls	Non-fatal MI	0.61	p >.05
Adam et al[32]	Case-control	76 cases 151 controls	Fatal MI	0.65	p >.05
Pfeffer et al[33]	Case-control	185 cases 511 controls	Fatal/non-fatal MI	0.68	p >.05
Rosenberg et al[34]	Case-control	336 cases 6,730 controls	Non-fatal MI	0.97	p >.05
Rosenberg et al[35]	Case-control	477 cases 1,832 controls	Non-fatal MI	1.00	p >.05
Jick et al[36]	Case-control	17 cases 34 controls	Non-fatal MI	7.5	p <.05
Lafferty et al[37]	Cohort	124 women	Fatal/non-fatal MI	0.16	p = .05
MacMahon[38]	Cohort	1,891 women	All CVD	0.30	NA
Stampfer et al[39]	Cohort	32,317 women	All CVD	0.30	p <.01
Hammond et al[40]	Cohort	610 women	All CVD	0.33	p <.01
Potocki et al[41]	Cohort	198 women	All CVD	0.33	NA
Bush et al[42]	Cohort	2,270 women	CVD mortality	0.34	p <.05
Burch et al[43]	Cohort	737 women	Fatal CHD	0.43	p <.05
Petitti et al[44]	Cohort	16,638 women	CVD deaths	0.50	p <.05
Henderson et al[45]	Cohort	7,610	Fatal/non-fatal MI	0.54	p <.05
Wilson et al[46]	Cohort	1,234 women	All CVD	1.76	p <.05

MI = myocardial infarction; CHD = coronary heart disease; CVD = cardiovascular disease; NA = not available.

with the placebo group, had a relative risk 0.33 for fatal and nonfatal myocardial infarction. The non-representativeness of the study subjects, the absence of data showing the success of randomization and the distribution of other medication use, and the small sample size all limit the conclusions that can be drawn from this single clinical trial.

Case-Control Studies

Of the eight case-control studies reported, five[29–33] show relative risks for heart disease among estrogen users to be between 0.33 and 0.68 that of non-users,

two showed no effect of estrogen use,[34,35] and one reported an increased risk for estrogen users.[36]

Both of the case-control studies that found no effect of estrogen therapy on heart disease were analyses done by Rosenberg and colleagues.[34,35] In their first report from the Boston Collaborative Drug Surveillance Program (1976), they compared estrogen use in 336 women between the ages of 40 and 75 years with non-fatal myocardial infarction with estrogen use in 6730 controls. They initially found a crude odds ratio of 0.47 for estrogen use. However, after adjusting for a wide variety of factors, including religion, hospital site, and coffee consumption, the odds ratio was found to be 0.97.

In their second report, they compared estrogen use in women aged 30 to 49 years admitted with non-fatal myocardial infarction to coronary care units in 155 U.S. hospitals with estrogen use in 1832 controls. The odds ratio for recent estrogen use was found to be 1.0, and that for past use was 1.2. The generalizability of these findings is unknown, inasmuch as women between the ages of 30 and 49 years are at very low risk of both estrogen replacement therapy and myocardial infarction.

The case-control study that reported an increased risk for estrogen users was reported by Jick and associates in 1978.[36] In this small study with 17 cases of non-fatal myocardial infarction and 34 controls, they found an odds ratio of 7.5 for estrogen use. However, they had initially identified 107 cases of myocardial infarction but were able to include only 17 in the analyses; additionally, 16 of 17 women were smokers. These serious methodologic problems make the results of this analysis questionable.

Cohort Studies

With the exception of the Framingham Study, all of the other nine cohort studies to date have found a protective effect of estrogen use on coronary heart disease. The relative risks reported have ranged from 0.16 to 0.54. Five of these major studies, including Framingham, are reviewed below.

THE NURSES STUDY. Stampfer and colleagues[39] surveyed by mail over 120,000 female nurses who were aged 30 to 55 years in 1976. At that time, baseline information on hormone use and other coronary risk factors was ascertained. Over 92 percent of the initial cohort was located via questionnaire in 1978 and 1980, and risk factor status and incident coronary disease were gathered at these times. The incidence of non-fatal myocardial infarction and fatal heart disease was then calculated for women who had never used postmenopausal hormones, for women who had ever used them, and for women who were currently using them at the baseline survey. Compared with never-users, ever-users had a relative risk of 0.5 for coronary disease, and current users had a relative risk of 0.3. These reductions in risk are statistically significant ($p < 0.01$). Statistical adjustment for reported smoking, hypertension, diabetes, hypercholesterolemia, family history of heart disease, past oral contraceptive use, and obesity did not alter the risk estimates. The authors conclude that their data support the hypothesis that postmenopausal estrogen use reduces the risk of coronary heart disease.

This study can be criticized because it relies almost entirely on self-report of risk factors, including hormone use. Such misclassification could bias the risk estimates. Nonetheless, the very large numbers of postmenopausal women ($N = 32,317$) and person-years of follow-up ($PY = 105,786$) mean that any random misclassification bias should not appreciably affect the results.

LEISURE WORLD. Henderson and associates[45] mailed a questionnaire in 1981 to all residents of Leisure World, Laguna Hills, an upper-middle-class retirement community near Los Angeles. Over 60 percent of the population responded, and this identifed cohort was enrolled in a mortality follow-up study. Follow-up includes all hospital admissions to the three hospitals serving the area and all deaths reported to the county health department. After 2 years, less than 1 percent of the 7610 women had been lost to follow-up.

After 3 years of follow-up, 56 deaths due to acute myocardial infarction were observed. The risk of death from myocardial infarction in ever-users of estrogens compared with never-users was 0.54. This finding is statistically significant ($p < 0.01$) and not influenced by previous history of heart attack or angina, hypertension, body weight, hysterectomy status, or smoking. The authors conclude that the finding of a protective effect of estrogen use from death from acute myocardial infarction is consistent with secular changes observed in death rates from MI. That is, the decline in cardiovascular mortality rates since 1960 is consistent with the increased use of estrogen since that time.

WALNUT CREEK. Petitti and coworkers[44] followed a group of 16,638 women, aged 18 to 54, who were members of The Northern California Kaiser-Permanente Medical Care Program. These women had been recruited into a study of contraceptive drug use in the late 1960s and early 1970s and provided data on all hormone use at entry. Women who had ever used oral contraceptives or who had a history of cardiovascular disease were excluded from the analysis. Mortality rates for all cardiovascular deaths was lower (RR = 0.80) in women reporting any noncontraceptive estrogen use. After statistically adjusting for other cardiovascular risk factors, including age, smoking, alcohol use, body mass, and history of hypertension, the relative risk of cardiovascular disease deaths in users compared with non-users was 0.50. This represents a statistically significant reduction in risk of cardiovascular mortality among estrogen users.

FRAMINGHAM. Wilson and associates,[46] using data gathered previously in the Framingham Heart Study, classified participants as estrogen users if that medication was recorded on their medication form at any of the biennial examinations 8 through 12. Additionally, participants had to be postmenopausal and 50 years of age or older at the 12th examination. A total of 1234 women met these criteria and were then followed for 8 years. Cardiovascular disease occurrence was defined to include all of the following: coronary heart disease, angina pectoris, myocardial infarction, stroke, transient ischemic attack, intermittent claudication, congestive heart failure, coronary death, and sudden death. All cardiovascular disease rates were significantly higher in women who reported any estrogen use. After adjustment for age, blood pressure, body mass, total cholesterol/HDL cholesterol, smoking and alcohol consumption, estrogen users compared with non-users had a relative risk of 1.76 ($p < 0.05$) for all cardiovascular disease. Deaths from all causes were not elevated (RR = 0.97). The authors conclude that estrogen therapy has potential drawbacks, particularly in regard to cardiovascular disease.

The inclusion of a wide variety of endpoints in the definition of cardiovascular disease is troubling and may lead to bias if, for example, a physician may be more likely to diagnose a transient ischemic attack in a woman taking estrogen. Furthermore, the statistical adjustment for the total cholesterol/HDL cholesterol ratio can be viewed as inappropriate, inasmuch as estrogen use both strongly influences these measures and exerts its putative protective effect by this mecha-

nism. A re-analysis of the Framingham data in women aged 50 to 60, using specific endpoints, and 10-year incidence rates and not adjusting for the cholesterol and lipoprotein ratio, has shown that the overall risk of coronary heart disease in estrogen users was approximately half that of non-users. It is difficult to assess the meaning of these discrepant results from the same data. Perhaps additional analyses from this cohort will be forthcoming.

LRC Follow-Up Study. Bush and colleagues[42] followed 2270 white women aged 40 to 69 at baseline for an average of 8½ years in the Lipid Research Clinics Follow-Up Study. Estrogen use was defined at one point (between 1972 and 1974), and the endpoint was death from all cardiovascular diseases. Cardiovascular deaths were defined by a mortality classification panel comprising five cardiologists. Follow-up of the participants was virtually complete. After 8½ years, women using estrogens, compared with non-users, had a relative risk of cardiovascular death of 0.34. This reduction in risk is statistically significant ($p < 0.05$) and was not influenced by adjustment for age, smoking, blood pressure, total cholesterol level, alcohol use, body mass, exercise, triglycerides, education, and hysterectomy status. However, adjustment for HDL and LDL cholesterol levels did markedly diminish the protective effect of estrogen use on cardiovascular death. The authors conclude that the protective effect of estrogen on cardiovascular disease death is mediated by increased HDL levels among estrogen users.

CONCLUSIONS

Unopposed estrogen replacement therapy appears to have a beneficial effect on lipoproteins and blood pressures and to be highly protective for the subsequent development of fatal and non-fatal coronary disease in women. Because the vast majority of these studies are observational, the issue of selection bias for estrogen use (i.e., healthier women are more likely to be prescribed estrogen) cannot be laid to rest. However, extensive post-hoc analyses in all of the cohort studies (with the exception of Framingham) reveal no apparent differences in cardiovascular risk between estrogen users and non-users. A randomized clinical trial to address the question of selection bias is probably warranted, although unlikely (owing to feasibility issues).

Perhaps the major unanswered question at this time is whether the use of estrogen cycled with a progestin is as protective against cardiovascular disease as is the use of unopposed estrogen. Currently, the data on the effects of estrogen/progestin formulations on coronary heart disease risk factors are mixed; and unfortunately, data on estrogen/progestin use and risk of actual heart disease are non-existent. However, given the popular current prescribing practices of both cyclic and continuous estrogen-progestin therapy, this question may be addressable in the near future.

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